ORIGINAL ARTICLE

Journal of Cachexia, Sarcopenia and Muscle 2019; **10**: 974–984
Published online 29 May 2019 in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/jcsm.12442

Association of lifestyle factors and inflammation with sarcopenic obesity: data from the PREDIMED-Plus trial

Itziar Abete[1,2,3†], Jadwiga Konieczna[2,4†], M. Angeles Zulet[1,2,3*], Aina M. Galmés-Panades[2,4], Idoia Ibero-Baraibar[1], Nancy Babio[2,5], Ramón Estruch[2,6], Josep Vidal[7,8], Estefanía Toledo[2,3,9], Cristina Razquin[3,9], Rafael Bartolomé[10], Andrés Díaz-Lopez[2,5], Miquel Fiol[2,4], Rosa Casas[2,6], Josep Vera[11], Pilar Buil-Cosiales[2,3,9,10], Xavier Pintó[2,12], Emili Corbella[2,12], Maria Puy Portillo[2,13], Jose Antonio de Paz[14], Vicente Martín[15,16], Lidia Daimiel[17], Albert Goday[18], Nuria Rosique-Esteban[2,5], Jordi Salas-Salvadó[2,5], Dora Romaguera[2,4], J. Alfredo Martínez[1,2,3,17] & on behalf of PREDIMED-PLUS Investigators

[1]*Department of Nutrition, Food Sciences and Physiology, Center for Nutrition Research, University of Navarra (UNAV), Pamplona, Spain,* [2]*CIBER Fisiopatología de la Obesidad y Nutrición (CIBERobn), Instituto de Salud Carlos III, Madrid, Spain,* [3]*IdiSNA (Instituto de Investigación Sanitaria de Navarra), Pamplona, Spain,* [4]*Instituto de Investigación Sanitaria Illes Balears (IdISBa), University Hospital Son Espases, Palma de Mallorca, Spain,* [5]*Department of Biochemistry and Biotechnology, Human Nutrition Unit, IISPV, Rovira i Virgili University, Hospital Universitari Sant Joan de Reus, Reus, Spain,* [6]*Department of Internal Medicine, IDIBAPS, Hospital Clinic, University of Barcelona, Barcelona, Spain,* [7]*Department of Endocrinology, IDIBAPS, Hospital Clinic, University of Barcelona, Barcelona, Spain,* [8]*CIBER Diabetes y enfermedades metabólicas (CIBERdem), Instituto de Salud Carlos III (ISCIII), Madrid, Spain,* [9]*Department of Preventive Medicine and Public Health, University of Navarra, Pamplona, Spain,* [10]*Atención Primaria, Servicio Navarro de Salud-Osasunbidea, Pamplona, Spain,* [11]*Institut Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain,* [12]*Vascular Risk Unit, Internal Medicine Department, Bellvitge University Hospital—IDIBELL, Hospitalet de Llobregat, Barcelona, Spain,* [13]*Nutrition and Obesity Group, Department of Nutrition and Food Science, University of the Basque Country (UPV/EHU) and Lucio Lascaray Research Institute, Vitoria, Spain,* [14]*Instituto de Biomedicina (IBIOMED), University of León, León, Spain,* [15]*Division of Preventive Medicine, University of León, León, Spain,* [16]*CIBER Epidemiología y Salud Pública (CIBEResp), Instituto de Salud Carlos III (ISCIII), Madrid, Spain,* [17]*Madrid Institute for Advanced Studies (IMDEA), Food Institute, Madrid, Spain,* [18]*Lipids and Cardiovascular Epidemiology Research Unit, Institut Municipal d'Investigació Mèdica (IMIM), Endocrinology and Diabetes Unit, Department de Medicina, Hospital del Mar Barcelona, Universitat Autònoma de Barcelona, Barcelona, Spain*

Abstract

Background Sarcopenia is a progressive age-related skeletal muscle disorder associated with increased likelihood of adverse outcomes. Muscle wasting is often accompanied by an increase in body fat, leading to 'sarcopenic obesity'. The aim of the present study was to analyse the association of lifestyle variables such as diet, dietary components, physical activity (PA), body composition, and inflammatory markers, with the risk of sarcopenic obesity.

Methods A cross-sectional analysis based on baseline data from the PREDIMED-Plus study was performed. A total of 1535 participants (48% women) with overweight/obesity (body mass index: 32.5 ± 3.3 kg/m^2; age: 65.2 ± 4.9 years old) and metabolic syndrome were categorized according to sex-specific tertiles (T) of the sarcopenic index (SI) as assessed by dual-energy X-ray absorptiometry scanning. Anthropometrical measurements, biochemical markers, dietary intake, and PA information were collected. Linear regression analyses were carried out to evaluate the association between variables.

Results Subjects in the first SI tertile were older, less physically active, showed higher frequency of abdominal obesity and diabetes, and consumed higher saturated fat and less vitamin C than subjects from the other two tertiles (all $P < 0.05$). Multiple adjusted linear regression models evidenced significant positive associations across tertiles of SI with adherence to the Mediterranean dietary score (P-trend < 0.05), PA (P-trend < 0.0001), and the 30 s chair stand test (P-trend < 0.0001), whereas significant negative associations were found with an inadequate vitamin C consumption (P-trend < 0.05), visceral fat and leucocyte count (all P-trend < 0.0001), and some white cell subtypes (neutrophils and monocytes), neutrophil-to-lymphocyte ratio, and platelet count (all P-trend < 0.05). When models were additionally adjusted by potential mediators (inflammatory markers, diabetes, and waist circumference), no relevant changes were observed, only dietary variables lost significance.

Conclusions Diet and PA are important regulatory mediators of systemic inflammation, which is directly involved in the sarcopenic process. A healthy dietary pattern combined with exercise is a promising strategy to limit age-related sarcopenia.

Keywords Sarcopenic index; Visceral fat; Leucocyte count; Mediterranean diet score; Systemic inflammation; Physical activity

Received: 24 September 2018; Accepted: 5 April 2019
**Correspondence to: M. Angeles Zulet, Department of Nutrition, Food Sciences and Physiology, Center for Nutrition Research, University of Navarra (UNAV), Pamplona, Spain. Phone: +34 948425600 ext 806317/fax, Email: mazulet@unav.es*
†Itziar Abete and Jadwiga Konieczna shared the first authorship.

Introduction

Sarcopenia is an age-associated process characterized by a progressive loss of skeletal muscle mass and strength.[1] This condition is a major health concern in older adults because it has been associated with metabolic impairments, cardiovascular disease risk factors, and physical and functional disability and increases the likelihood/risk of early mortality.[2] All these metabolic and physical alterations cause important healthcare costs and significantly affect quality of life.[3] Sarcopenia often coexists with obesity leading to a specific condition named 'sarcopenic obesity'.[4] Current evidences suggest that sarcopenic obesity may be associated with a larger number of metabolic disorders and an increased risk of mortality than obesity or sarcopenia alone.[5] However, few studies have been carried out in this field and with contradictory results. Cross-sectional studies in sarcopenic obesity subjects have reported higher prevalence of cardiovascular risk factors and metabolic syndrome in those individuals.[6,7] Strong associations with inflammatory markers compared with sarcopenia-only subjects have been found.[8] Conversely, some longitudinal studies have shown that sarcopenic obesity does apparently not confer any greater mortality risk than sarcopenia alone.[9–11]

There is evidence that the biological process of ageing is characterized by oxidative stress and mitochondrial dysfunction.[12] Increased reactive oxygen species production and decreased antioxidant defences in older people seem to be important factors contributing to muscle impairment.[13] During ageing, in turn, chronic low-grade inflammation, called inflammaging, develops, which contributes to the pathogenesis of age-related diseases.[14] Inflammaging has been described as a common biological component of main age-related chronic diseases such as atherosclerosis and type 2 diabetes mellitus, and age-related conditions like sarcopenia, frailty, and disability.[15] Another new concept that has emerged is 'metaflammation', the metabolic inflammation accompanying metabolic diseases driven by nutrient excess or overnutrition; metaflammation is characterized by the same mechanisms underpinning inflammaging. Lifestyle factors like diet and physical activity (PA) are able to modulate oxidative and inflammatory processes.[16] Improving nutrition has been proposed as an effective strategy to reduce inflammaging and to prevent or decelerate diet-related and age-related diseases.[17,18] Therefore, it could be hypothesized that lifestyle could play an important role in the management and reversion of muscle mass loss and promote a healthy body composition (BC). In fact, PA (resistance training) in combination with proper nutrition (protein intake) is a promising strategy to limit age-related sarcopenia.[19] Nonetheless, little information is available comparing nutrient intake, BC, and lifestyle between sarcopenic and non-sarcopenic obese older adults as well as about the role of inflammatory processes associated with sarcopenia. The aim of the present study was to analyse the association of lifestyle variables such as diet, dietary components, PA, BC, and inflammatory markers, with the risk of sarcopenic obesity.

Materials and methods

Study design

The current research is a cross-sectional analysis including baseline data of the PREDIMED-Plus study, a 6 year multicentre, randomized, parallel-group, primary prevention clinical trial conducted in Spain to assess the effect on cardiovascular disease morbi-mortality of a weight loss intervention programme based on an energy-restricted traditional Mediterranean diet, PA promotion, and behavioural support, in comparison with a usual care intervention only with energy-unrestricted Mediterranean diet (control group). A more detailed description of the PREDIMED-Plus study has been recently published,[20] and there is available study information at http://predimedplus.com/. This study was registered at the International Standard Randomized Controlled Trial (http://www.isrctn.com/ISRCTN89898870) with Number 89898870 (registration date 24 July 2014).

Study subjects

A total of 6874 participants were recruited at 23 different research sites in Spain and randomized to one of the two study groups (September 2013–December 2016). The eligible participants were community-dwelling adults (aged 65.2 ± 4.9 years) with overweight/obesity [body mass index (BMI) = 32.5 ± 3.3 kg/m^2], who met at least three components of the metabolic syndrome according to the updated harmonized criteria of the International Diabetes Federation and the American Heart Association and National Heart, Lung and Blood Institute.[21] Information about exclusion criteria has been previously published.[20] Most of participants (97.5%) were of Caucasian origin. All participants provided written informed consent, and the study protocol and procedures were approved according to the ethical standards of the Declaration of Helsinki by all the participating institutions.

The present work encompasses a subsample of participants who underwent dual-energy X-ray absorptiometry (DXA) scans for BC assessment (n = 1535) in seven out of the 23 PREDIMED-Plus recruiting centres, as these were the only centres with available DXA scanner for this research.

Assessment of body composition with dual-energy X-ray absorptiometry

Dual-energy X-ray absorptiometry scans (Lunar iDXA and DXA Lunar Prodigy Primo, GE Healthcare) were performed by trained radiology technicians to assess BC following a validated standardized protocol and subject positioning provided by the manufacturer. The DXA was calibrated daily according to manufacturer guidelines. Thus, total bone mass, fat mass, lean mass, fat-free mass, and regionally distributed fat and lean mass (trunk, android, gynoid, arms, and legs) were determined. For visceral adipose tissue measures in the android region, scans were reanalysed using validated CoreScan software application.[22] Appendicular skeletal muscle mass (ASM, kg) was calculated as the sum of the muscle mass from the four limbs as described elsewhere.[23]

Calculation of sarcopenic indexes

Skeletal muscle mass index (kg/m^2) was calculated with the equation ASM (kg)height^2 (m). The sarcopenic index (SI) was obtained by dividing the amount of ASM (kg) by the body weight (kg) × 100.[24] Participants were categorized according to the sex-specific SI tertiles (women: T_1: <21.0%, T_2: ≥21.0 to <22.7%, and T_3: ≥22.7; men: T_1: <26.3%, T_2: ≥26.3 to <28.5%, and T_3: ≥28.5%), which provide a good estimate of the amount of skeletal muscle mass relative to body size.[25] Thus, the higher index, the better for health.

Anthropometric, clinical, and biochemical variables

The anthropometric variables were measured at the baseline visit by trained staff according to the PREDIMED-Plus internal procedures. Body weight (kg) and height (cm) were measured in light clothing and without shoes with the use of a calibrated scale and a wall-mounted stadiometer, respectively. BMI was calculated as weight (kg) divided by the square of height (m). Waist circumference (cm) was measured midway between the lowest rib and the iliac crest using a measuring tape. Blood pressure and heart rate were measured in triplicate with the use of a validated semiautomatic oscillometer (Omron HEM-705CP, The Netherlands) according to World Health Organization criteria. Hypertensive participants were defined as those that registered high blood pressure levels (≥130/85 mmHg) at baseline or were on blood pressure medication.

Blood samples were collected at baseline after 12 h overnight fast and were used to perform biochemical analyses by means of standard laboratory enzymatic methods. These analyses included plasma glucose (mg/dL), glycated haemoglobin (HbA1c, %), high-density lipoprotein cholesterol (HDL-c, mg/dL), and triglyceride (mg/dL) levels. Current diabetes was defined as previous diagnosis of diabetes or HbA1c ≥ 6.5%, use of antidiabetic medication, or fasting glucose >126 mg/dL in the screening visit plus fasting glucose >126 mg/dL at baseline visit. Participants with low HDL-c concentration were those who had low HDL-c levels, defined as <40 mg/dL in men and <50 mg/dL in women, or with HDL-c medication at baseline. Hypertriglyceridaemia was defined as presence of high triglyceride levels (≥150 mg/dL) or triglyceride-lowering drugs. A complete blood count was also performed following standardized procedures. Blood parameters assessed in this study were the white blood cell count, its subtypes (lymphocytes, monocytes, neutrophils, and eosinophils), and platelet count.

Dietary variables

At baseline, a trained dietitian administered a validated 143-item semi-quantitative food frequency questionnaire to determine dietary factors in a face-to-face visit.[26] Participants were asked about their frequency consumption of each specific item during the preceding year. There were nine possible answers ranging from never to more than six times per day, which were transformed to g/day, taking into account the standard portion size of each item. Two Spanish food composition tables were used to calculate total energy and nutrients intake.[27,28]

The Mediterranean dietary pattern was calculated according to a validated Mediterranean dietary score,[29] considering the consumption of nine food groups or nutrients (cereals, fruits and nuts, vegetables, legumes, fish, meat, dairy products, ratio of monounsaturated to saturated fatty acids, and alcohol).

Physical activity variables

Leisure-time PA at baseline was evaluated with the validated REGICOR questionnaire as detailed elsewhere,[30] which included questions about the activity type, frequency (number of days), and duration (min/day) performed during a representative month. As described previously,[31] time spent in PA (min/day) was further obtained by assigning to each activity their corresponding intensity according to the compendium of PA.[32,33] Time spent in total PA was computed as the sum of the time from all PA intensities. The 30 s chair stand test was used as an indicator of the lower-limb muscle strength. As reported, performance was based on the number of times participants stand and sit in a chair in 30 s.[34]

Other sociodemographic variables

Participants self-reported baseline age, sex, smoking habit, and educational level. Smoking was categorized as current, former, and never smokers. Education was categorized as bachelor's degree, primary/secondary school, and no education/no data.

Statistical analyses

Normality of variables was initially studied by using the Shapiro–Wilk test. Baseline characteristics of the study participants are presented as means ± standard deviation for quantitative variables and numbers and percentages for categorical variables. Differences in anthropometric, BC, and biochemical variables, dietary characteristics, and food group intake among the three SI sex-specific tertiles were tested by analysis of variance and the χ^2 test for categorical variables.

Multiple adjusted linear regression models were used to evaluate the association between the variables showing a strong relationship (in tertiles) with SI (as continuous variable). We run first a minimally adjusted Model 1 including sex, age, and centre (all exposures). Model 2 was adjusted by the minimally sufficient adjustment set, determined using directed acyclic graphs (DAGs) implemented in DAGitty software,[35] available free on www.dagitty.net. The DAGs were built by identifying known factors affecting each of our exposures on SI (see DAGs in Supporting Information, *Figure* S1A–D). Thus, assuming the total effect of our exposures on SI, the covariables used in Model 2 included sex, age, centre, PA (in models with dietary, BC, and inflammatory variables as independent variables), Mediterranean dietary score (in models with PA, BC, and inflammatory variables as independent variables), and waist circumference (in models with inflammatory variables as independent variables).

Additionally, as sensitivity analyses, multivariable models (Model 3) adjusted for the same covariables as in Model 2 plus mediators (direct effect determined using DAGs) were run: diabetes, neutrophil-to-lymphocyte ratio (NLR) (in models with dietary, PA, and BC variables as independent variables), and waist circumference (in models with dietary and PA variables as independent variables).

Lastly, we evaluated plausible effect modification by age group (<65 or ≥65 years of age), sex, diabetes (yes/no), obesity prevalence (BMI $>$ 30 kg/m^2), and centre, by adding an interaction term between these variables and all exposures in a multiple adjusted linear regression model (Model 2). When a significant interaction was detected, stratified analyses were conducted.

Statistical analyses were performed using Stata 12.0, and the statistical significance was set at $P < 0.05$ for bilateral contrast.

Results

Subjects were categorized according to SI sex-specific tertiles. An overview on clinical and sociodemographic data, BC and inflammatory markers, and dietary characteristics, considering SI tertiles, are given in *Tables* 1, 2, and 3, respectively. Participants from the first SI tertile were on average older, shorter, and had higher BMI, heart rate, and blood glucose level than subjects from the upper two tertiles (all $P < 0.05$) (*Table* 1). Subjects in the first SI tertile also showed higher frequency of abdominal obesity and diabetes but lower frequency of low HDL-c and hypertriglyceridaemia than subjects from the upper two tertiles (all $P < 0.05$) (*Table* 1). No differences in smoking habit and educational level were observed among groups. On the other hand, significant differences were found in PA among SI tertiles ($P < 0.0001$) (*Table* 1). The 30 s chair test scoring was lower in participants from the first tertile as compared with subjects from the second and third tertiles ($P < 0.0009$) (*Table* 1).

Body composition variables showed no differences in bone mass between groups. Subjects in the first tertile had higher total fat mass, as well as higher trunk, android, gynoid, and visceral fat content than subjects from the second and third tertiles (all $P < 0.0001$) (*Table* 2). Regarding lean muscle mass, subjects in the first SI tertile had lower total lean mass and lower skeletal muscle mass index and fat-free mass content than subjects from the other groups (all $P < 0.001$) (*Table* 2).

No significant differences were observed in total energy intake among SI tertiles. However, subjects from the first tertile consumed less carbohydrate, fibre, and vitamin C and more saturated fat, than subjects from the other two tertiles (all $P < 0.05$) (*Table* 3). When dietary food groups were assessed, main differences were observed in fruits, cereals, and extra virgin olive oil, whose consumption was decreased in participants with lower SI (all $P < 0.05$) (*Table* 3).

Table 1 Clinical and sociodemographic characteristics of the study participants according to sex-specific SI tertiles (T)

	SI (%)			P-value
	T_1	T_2	T_3	
Men: 801	<26.3%	≥26.3 to <28.5%	≥28.5%	
Women: 734	<21.0%	≥21.0 to <22.7%	≥22.7%	
n = 1535	512	513	510	—
Men/women	267/245	268/245	266/244	—
Clinical variables				
Age (years)	65.6 ± 5.0	65.3 ± 4.9	64.8 ± 5.0	0.036
Weight (kg)	90.2 ± 13.1	85.9 ± 12.6	82.8 ± 11.7	<0.0001
Height (cm)	162.0 ± 9.1	163.5 ± 9.5	163.6 ± 9.3	0.019
BMI (kg/m^2)	34.2 ± 3.3	32.3 ± 3.0	30.8 ± 2.6	<0.0001
SBP (mmHg)	139.7 ± 16.9	140.1 ± 16.8	139.8 ± 16.8	0.940
DBP (mmHg)	79.9 ± 10.7	80.3 ± 9.8	80.3 ± 10.5	0.804
HR (b.p.m.)	70.9 ± 11.0	69.6 ± 10.8	67.6 ± 10.3	<0.0001
Glucose (mg/dL)	115.4 ± 32.6	112.1 ± 25.6	110.3 ± 23.9	0.010
Glycated haemoglobin (%)	6.10 ± 0.90	6.10 ± 0.70	6.00 ± 0.70	0.050
Metabolic syndrome criteria				
Abdominal obesity, *n* (%)	504 (98)	480 (94)	455 (89)	<0.0001*
Diabetes, *n* (%)	200 (39)	163 (32)	153 (30)	0.021*
Hypertension, *n* (%)	476 (93)	479 (93)	468 (92)	0.539*
Low HDL-c, *n* (%)	203 (40)	249 (49)	257 (50)	0.001*
Hypertriglyceridaemia, *n* (%)	251 (49)	298 (58)	313 (61)	<0.0001*
Sociodemographic variables				
Smoking habit, *n* (%)				
Never	230 (45)	208 (41)	211 (41)	0.523*
Current	64 (12)	72 (14)	56 (11)	
Former	218 (43)	231 (45)	243 (48)	
Family history of obesity (%)	289 (57)	272 (53.2)	243 (48.0)	0.016*
Educational level, *n* (%)				
Bachelor's degree	104 (20)	113 (22)	110 (22)	0.930*
Primary/secondary	403 (79)	396 (77)	396 (77)	
No education/no data	6 (1)	4 (1)	5 (1)	
Physical activity (MET, min/day)				
Inactive	329 (64)	274 (53)	245 (48)	<0.0001*
Moderately active	85 (17)	100 (19)	130 (26)	
Active	98 (19)	139 (27)	134 (26)	
30 s chair test (repeats)	13.5 ± 5.1	14.3 ± 5.3	14.7 ± 5.2	0.0009

BMI, body mass index; DBP, diastolic blood pressure; HR, heart rate; HDL-c, high-density lipoprotein cholesterol; MET, metabolic equivalent; SBP, systolic blood pressure; SI, sarcopenic index.
SI was calculated as a percentage of the total body weight: SI = [appendicular skeletal muscle mass (kg)weight (kg)] × 100. Data are presented as means ± standard deviations for quantitative variables and number and percentages for categorical variables. Differences in characteristics among the three SI sex-specific tertiles were tested by analysis of variance. Metabolic syndrome criteria: abdominal obesity: men: waist circumference ≥102 cm and women: waist circumference ≥88 cm; diabetes: fasting glucose ≥100 mg/dL or glycated haemoglobin ≥6.5% or taking treatment; hypertension: SBP ≥ 135 mmHg or DBP ≥ 85 mmHg or taking antihypertensive medication; low HDL-c levels: men ≤40 mg/dL and women ≤50 mg/dL or taking specific medication to regulate HDL-c; and hypertriglyceridaemia: fasting triglyceride concentration ≥150 mg/dL or taking medication to regulate triglycerides.
*Categorical variables: the *P*-value was obtained by means of the χ^2 test.

Moreover, lower adherence to a validated Mediterranean dietary score was observed in the first tertile group as compared with the second and third tertile participants (P = 0.001) (*Table* 3).

Linear regression models were set up with SI as the dependent variable and lifestyle variables such as diet, PA, BC, and inflammatory markers as independent factors (*Table* 4). Both minimally adjusted (Model 1) and multiple adjusted (Model 2) models showed significant positive associations between the lowest to highest tertile of SI and adherence to the Mediterranean dietary score (P-trend = 0.045), PA (P-trend $<$ 0.0001), and the 30 s chair stand test (P-trend $<$ 0.0001), whereas significant negative associations were found with an inadequate vitamin C consumption (P-trend = 0.018), visceral fat (P-trend $<$ 0.0001), leucocyte count (P-trend $<$ 0.0001), and some white cell subtypes [neutrophils and monocytes (P-trend = 0.012)], NLR (P-trend = 0.010), and platelet count (P-trend = 0.020). In Supporting Information, *Table* S1 are shown other independent variables that were significantly associated to SI in descriptive analyses. Carbohydrates, fruits intake, and lean mass were significantly and positively associated with SI (all P-trend $<$ 0.05), whereas saturated fat consumption, total fat, gynoid fat, and eosinophil count were inversely associated with SI (all P-trend $\leq$ 0.01). In order to estimate the magnitude of the association explained by the interrelation between lifestyle, obesity, inflammation, and sarcopenia, an additional Model 3 including all covariates to estimate the direct effect of

Table 2 Body composition by DXA scanning and inflammatory markers of study participants according to SI sex-specific tertiles (T)

	SI (%)			*P*-value
	T_1	T_2	T_3	
Men	<26.3%	≥26.3 to <28.5%	≥28.5%	
Women	<21.0%	≥21.0 to <22.7%	≥22.7%	
Body composition variables				
Bone mass (kg)	2.6 ± 0.5	2.6 ± 0.5	2.6 ± 0.5	0.255
Total fat mass (%)	43.8 ± 6.0	40.3 ± 6.3	36.5 ± 6.5	<0.0001
Trunk fat (kg)	23.2 ± 4.3	20.1 ± 3.5	17.4 ± 2.9	<0.0001
Android fat (kg)	4.3 ± 0.9	3.6 ± 0.7	3.1 ± 0.6	<0.0001
Gynoid fat (kg)	5.7 ± 1.4	5.0 ± 1.3	4.2 ± 1.0	<0.0001
VAT (kg)	2.6 ± 0.9	2.3 ± 0.8	2.0 ± 0.6	<0.0001
Total lean mass (%)	53.1 ± 5.8	56.5 ± 6.0	60.1 ± 6.2	<0.0001
SMI (kg/m^2)	7.6 ± 1.0	8.0 ± 1.1	8.4 ± 1.1	<0.0001
FFM (kg)	49.8 ± 9.8	50.8 ± 10.3	52.2 ± 10.6	0.0008
Inflammatory markers				
WBC (× 10^9/L)	6.9 ± 1.8	6.8 ± 2.0	6.5 ± 3.2	0.043
Lymphocytes	2.5 ± 3.5	2.6 ± 3.8	2.4 ± 3.3	0.844
Monocytes	0.6 ± 0.7	0.5 ± 0.7	0.5 ± 0.7	0.908
Neutrophils	4.8 ± 6.7	4.5 ± 6.3	4.2 ± 6.3	0.434
Eosinophils	0.2 ± 0.4	0.2 ± 0.2	0.2 ± 0.2	0.236
NLR	2.2 ± 3.4	2.0 ± 1.6	1.8 ± 1.6	0.072
Platelet count (× 10^9/L)	230 ± 57	227 ± 54	226 ± 57	0.440

DXA, dual-energy X-ray absorptiometry; FFM, fat-free mass; NLR, neutrophil-to-lymphocyte ratio; VAT, visceral adipose tissue; SI, sarcopenic index; SMI, skeletal muscle mass index; WBC, white blood cell count.
Differences in characteristics among the three SI sex-specific tertiles were tested by analysis of variance.

Table 3 Dietary consumption of study participants according to SI sex-specific tertiles (T)

	SI (%)			*P*-value
	T_1	T_2	T_3	
Men: 803	<26.3%	≥26.3 to <28.5%	≥28.5%	
Women: 734	<21.0%	≥21.0 to <22.7%	≥22.7%	
Nutrients				
Energy intake (kcal/day)	2402 ± 582	2389 ± 576	2443 ± 589	0.293
Carbohydrates (%)	39.6 ± 6.4	40.0 ± 6.7	40.9 ± 6.2	0.003
Proteins (%)	16.4 ± 2.6	16.4 ± 2.7	16.1 ± 2.4	0.094
Lipids (%)	40.6 ± 6.1	40.2 ± 6.2	39.7 ± 5.8	0.052
PUFA (%)	6.2 ± 1.8	6.3 ± 1.8	6.2 ± 1.7	0.553
SFA (%)	10.3 ± 1.9	10.0 ± 1.9	9.8 ± 1.9	0.0001
MUFA (%)	21.3 ± 4.5	21.0 ± 4.3	20.8 ± 4.0	0.238
Fibre (g/day)	25.2 ± 8.3	25.4 ± 9.0	26.8 ± 8.2	0.003
Alcohol intake (g/day)	11.6 ± 15.2	11.8 ± 15.5	11.7 ± 15.1	0.974
ω_3 fatty acids (g/day)	0.85 ± 0.44	0.86 ± 0.45	0.88 ± 0.46	0.578
Fish ω_3 fatty acids (g/day)	0.63 ± 0.33	0.63 ± 0.35	0.66 ± 0.36	0.312
Vitamin C (mg/day)	194 ± 81	202 ± 89	209 ± 84	0.016
Vitamin D (μg/day)	5.8 ± 3.1	5.8 ± 3.2	6.0 ± 3.3	0.610
Food groups				
Vegetables (g/day)	300 ± 116	308 ± 127	318 ± 122	0.054
Fruits (g/day)	321 ± 193	328 ± 192	366 ± 206	0.0004
Legumes (g/day)	20.0 ± 11.4	19.6 ± 10.4	19.5 ± 9.6	0.686
Cereals (g/day)	146 ± 79	153 ± 84	163 ± 83	0.004
Dairy products (g/day)	345 ± 215	336 ± 206	329 ± 193	0.418
Meats (g/day)	157 ± 59	155 ± 61	149 ± 56	0.087
Extra virgin olive oil (g/day)	31.5 ± 21.0	33.9 ± 19.9	34.6 ± 19.8	0.038
Fish (g/day)	95.9 ± 42.3	95.8 ± 45.2	101 ± 47	0.150
Nuts (g/day)	14.0 ± 17.9	14.2 ± 16.0	14.9 ± 17.1	0.649
Confectionery (g/day)	26.5 ± 33.5	26.3 ± 28.5	28.1 ± 34.1	0.606
Mediterranean diet score (points)	4.2 ± 1.5	4.3 ± 1.6	4.5 ± 1.6	0.001

MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; SI, sarcopenic index.
Differences in characteristics among the three SI sex-specific tertiles were tested by analysis of variance.

Table 4 Linear regression models with SI as the dependent variable and lifestyle, body composition, and inflammatory variables as independent factors

		Model 1, β (95%CI)	Model 2, β (95%CI)	Model 3, β (95%CI)
Dietary variables				
Mediterranean diet score (points)	T_1	Ref	Ref	Ref
	T_2	0.02 (–0.25; 0.30)	−0.01 (−0.27; 0.26)	−0.14 (−0.39; 0.09)
	T_3	0.39 (0.09; 0.69)	0.32 (0.02; 0.62)	0.16 (−0.10; 0.42)
	P-trend	0.015	0.045	0.340
Vitamin C (mg/day)	≥RDA	Ref	Ref	Ref
	<RDA	−0.65 (−1.18; −0.11)	−0.55 (−1.09; −0.02)	−0.64 (−1.12; −0.16)
	P-trend	0.018	0.041	0.009
Physical activity variables				
Physical activity (MET, min/day)	Inactive	Ref	Ref	Ref
	Moderately active	0.84 (0.55; 1.14)	0.82 (0.52; 1.11)	0.48 (0.21; 0.75)
	Active	1.11 (0.79; 1.43)	1.08 (0.76; 1.41)	0.59 (0.29; 0.89)
	P-trend	<0.0001	<0.0001	<0.0001
30 s chair test (repeats)	T_1	Ref	Ref	Ref
	T_2	0.49 (0.21; 0.77)	0.49 (0.21; 0.76)	0.24 (−0.01; 0.49)
	T_3	1.04 (0.72; 1.36)	1.02 (0.70; 1.35)	0.55 (0.25; 0.84)
	P-trend	<0.0001	<0.0001	<0.0001
Body composition variables				
Android fat (g)	T_1	Ref	Ref	Ref
	T_2	−1.45 (1.69; −1.21)	−1.43 (−1.67; −1.19)	−1.42 (−1.66; −1.18)
	T_3	−2.91 (−3.15; −2.67)	−2.85 (−3.10; −2.61)	−2.83 (−3.07; −2.58)
	P-trend	<0.0001	<0.0001	<0.0001
Visceral adipose tissue (g)	T_1	Ref	Ref	Ref
	T_2	−0.72 (−1.14; −0.30)	−0.70 (−1.07; −0.33)	−0.68 (−1.05; −0.30)
	T_3	−1.88 (−2.30; −1.46)	−1.81 (−2.19; −1.44)	−1.81 (−2.19; −1.43)
	P-trend	<0.0001	<0.0001	<0.0001
Inflammatory variables				
Leucocyte count ($\times 10^9$/L)	T_1	Ref	Ref	Ref
	T_2	−0.21 (−0.49; 0.05)	−0.26 (−0.51; −0.01)	−0.22 (−0.47; 0.02)
	T_3	−0.69 (−0.97; −0.41)	−0.45 (−0.71; −0.20)	−0.45 (−0.70; −0.20)
	P-trend	<0.0001	<0.0001	0.001
Neutrophil count ($\times 10^9$/L)	T_1	Ref	Ref	Ref
	T_2	−0.31 (−0.59; −0.03)	−0.24 (−0.49; 0.007)	−0.22 (−0.47; 0.02)
	T_3	−0.75 (−1.03; −0.48)	−0.48 (−0.73; −0.23)	−0.45 (−0.70; −0.20)
	P-trend	<0.0001	0.012	<0.0001
Monocyte count ($\times 10^9$/L)	T_1	Ref	Ref	Ref
	T_2	−0.38 (−0.66; −0.10)	−0.26 (−0.51; −0.009)	−0.23 (−0.49; 0.01)
	T_3	−0.59 (−0.88; −0.30)	−0.32 (−0.59; −0.06)	−0.29 (−0.55; −0.02)
	P-trend	<0.0001	0.012	0.026
NLR	T_1	Ref	Ref	Ref
	T_2	−0.0006 (−0.27; 0.27)	0.08 (−0.16; 0.33)	0.08 (−0.16; 0.33)
	T_3	−0.52 (−0.80; −0.23)	−0.33 (−0.58; −0.08)	−0.31 (−0.57; −0.06)
	P-trend	<0.0001	0.010	0.015
Platelet count ($\times 10^9$/L)	T_1	Ref	Ref	Ref
	T_2	−0.06 (−0.34; 0.21)	−0.13 (−0.38; 0.11)	−0.13 (−0.38; 0.11)
	T_3	−0.36 (−0.64; −0.08)	−0.30 (−0.55; −0.04)	−0.28 (−0.53; −0.30)
	P-trend	0.012	0.020	0.028

CI, confidence interval; MET, metabolic equivalent; NLR, neutrophil-to-lymphocyte ratio; RDA, recommended dietary allowances; SI, sarcopenic index.
RDA for vitamin C (90 mg/day for men and 75 mg/day for women). Analyses were performed using multivariable linear regression models. Model 1: adjusted by centre, sex, and age; Model 2: Model 1 additionally adjusted for plausible confounders: physical activity (in models with dietary, body composition, and inflammatory variables as independent variables), Mediterranean dietary score (in models with physical activity, body composition, and inflammatory variables as independent variables), and waist circumference (in models with inflammatory variables as independent variables); and Model 3 (sensitivity analyses): Model 2 additionally adjusted by plausible mediators: diabetes, NLR (in models with dietary, physical activity, and body composition variables as independent variables), and waist circumference (in models with dietary and physical activity variables as independent variables).

each exposure on SI, independently of potential mediators (*Table* 4 and Supporting Information, *Table* S1), was performed. Overall, no relevant changes were detected, that is, associations that were significant in Model 2 remained significant in Model 3. The association of some dietary exposures, that is, Mediterranean diet score, carbohydrates, and fruits intake, became weaker after adjusting for diabetes, obesity, and inflammation.

Effect modification analyses revealed a significant interaction between sex and PA, android fat, neutrophil, and platelet count on SI (all *P* for interaction <0.05). However, analyses stratified by sex showed that only the association between platelet count and SI was significant in men (*P*-trend = 0.002) but not in women (*P*-trend = 0.687). Also, the association between NLR and SI was only statistically evident in diabetics (*P*-trend = 0.027, *P* for interaction = 0.029) (Supporting Information, *Table* S2). Centre heterogeneity was observed in the association between the android fat, 30 s chair test, leucocyte count, and NLR with SI (all $P < 0.05$) (Supporting Information, *Figure* S2A–D).

Discussion

The aim of the present study was to analyse the association of lifestyle variables such as diet, dietary components, PA, BC, and inflammatory markers, with the risk of sarcopenia. Most relevant results show that diet, the adherence to a dietary pattern such as Mediterranean diet as well as some dietary components like vitamin C and saturated fat, and PA are associated with sarcopenia, while obesity, particularly abdominal obesity, some metabolic alterations such as diabetes, and inflammatory factors seem to be mediating the effect. Both diet and PA have a direct impact on the development of obesity and metabolic alterations. This effect might be mediated mainly through the regulation of the inflammatory state as observed in the regression Model 3 when potential mediators were included. The association of diet with the SI lost the significance when the model was additionally adjusted by waist circumference, NLR, and diabetes, which suggests that obesity, inflammation, and diabetes are strong mediators of the effect of diet on SI. Interestingly, some specific dietary components such as vitamin C and saturated fat remained significant after adjusting by these potential mediators. Vitamin C and saturated fat consumption could have a direct effect on the process of sarcopenia. Some authors suggest that micronutrient intake, specifically vitamin C, is inadequate in obese subjects and subjects with metabolic syndrome.[36,37] An inadequate intake of vitamin C contributes to small overgrowth, transcytosis of enteric bacteria, and an elevation of lipopolysaccharides, which elicits a low-grade inflammatory response.[37] On the other hand, saturated fat can activate transmembrane proteins (toll-like receptors 2 and 4) involved in the activation of the innate immune system, leading to the activation of proinflammatory pathways and the secretion of proinflammatory cytokines, which predispose to adipose tissue inflammation and subsequent insulin resistance.[38]

The sarcopenia condition of the study participants could be assumed as a chronic condition considering that all subjects of the study were overweight or obese and had metabolic syndrome. Chronic sarcopenia is likely to be associated with permanent and progressive processes such as ageing, obesity, and metabolic syndrome.[1,39] It is not established which condition develops first, and due to the cross-sectional nature of the study, it is not possible to determine the cause and consequence. Sarcopenia and obesity are considered as the multifactorial syndromes with various overlapping causes and feedback mechanisms supposed to be strongly interconnected and aggravate each other.[40] On the other hand, the natural process of ageing is associated with an increase in visceral fat and a progressive loss of muscle mass driven by a low-grade chronic inflammation also referred as 'inflammaging'.[14] The excess of android and visceral fat promotes inflammation leading to a low-grade chronic inflammatory state that is involved in the obesity-related metabolic alterations as well as in the process of sarcopenia. Participants in the study had a leucocyte count within the clinical range; however, the slight and clinically insignificant increase in leucocytes observed in subjects from tertile 1 might be accelerating the sarcopenic process. An accelerated or decelerated ageing will depend on the individual genetic background interacting lifelong with environmental and lifestyle factors (nutrition, physical, mental activity, etc.).[14] The metabolic pathways involved in sarcopenia and sarcopenic obesity are poorly understood. However, available scientific evidences support the role of inflammation on the process of sarcopenia, which seem to be mediated through alterations on glucose metabolism such as hyperglycaemia and insulin resistance.[8,41] Insulin resistance often occurs with abdominal obesity, and the association between both conditions is influenced by an overproduction of inflammatory cytokines.[42,43] Hyperglycaemia, insulin resistance, and excess cytokine production have been shown to impact sarcopenia by promoting the deterioration of skeletal muscle fibre diameter and protein content, as well as prompting metabolic breakdown of skeletal muscle encouraging sarcopenic process.[44] In the present study, a significant interaction between NLR and diabetes on the SI was featured. The stratified analysis (diabetics and no diabetics) showed that the association of NLR with the SI was significant only in diabetic participants according to the theory that insulin resistance and inflammation impact on the sarcopenia process.

Several sex interactions were found with PA, android fat, and neutrophil and platelet counts on SI. In the stratified analysis of the data by sex, the association of these exposures was not different between men and women, simply was stronger in men than in women. Men have more appendicular skeletal muscle mass, and the PA could be more effective in men than in women. On the other hand, android fat mass content used to be greater in men than in women. Therefore, inflammatory markers as well as android fat mass content were more strongly associated to SI in men than in women. Centre interactions were also observed with the 30 s chair test, android fat, mass content, leucocyte count, and NLR on the risk of SI. It is important to mention that despite the

DOI: 10.1002/jcsm.12442

establishment of a standardized protocol and the great efforts of all the groups involved in the project to follow it correctly, it has finally been inevitable to obtain some heterogeneity between centres. Accordingly, we repeated the analyses stratifying by centre. Some centres showed higher spread data, but the associations were in the same direction. In any case, care should be taken concerning the overall interpretation of the data.

Some limitations should be mentioned concerning this investigation, because it is a cross-sectional design and causal effects cannot be inferred, and although regression models were adjusted for many variables, residual confounding is still possible. Depending on the sarcopenic obesity definition, there are more or less subjects with sarcopenic obesity, which indicates that some definitions could be underestimating/overestimating sarcopenia. Nutritional information and PA data come from self-reported questionnaires, and in spite that FFQ and PA questionnaires have been previously validated, this information should be treated with caution.

Conclusions

The present research investigated main differences in BC, biochemical, and lifestyle variables among pre-obese and obese older adults with metabolic syndrome and different SI values, trying to find out risk factors potentially involved in the onset and development of sarcopenia/sarcopenic obesity. Important differences were observed in BC, biochemical, and lifestyle factors being android/visceral adipose tissue, leucocyte and platelet counts, and NLR strong risk factors for the SI, while adherence to the Mediterranean diet, the intake of some specific nutrients (such as vitamin C), and PA appeared as protective factors for the development of sarcopenia. Improving nutrition and regular PA are key factors for decelerating the sarcopenic process and assure a healthy ageing. Actions to develop successful intervention programmes for a healthy lifestyle in order to promote a quality ageing focused on muscle mass maintenance, prevention of obesity, and independence living are warranted.

Acknowledgements

We thank all the volunteers for the participation and personnel for their contribution in the PREDIMED-PLUS trial.

The authors certify that they comply with the ethical guidelines for authorship and publishing of the *Journal of Cachexia*, *Sarcopenia and Muscle*.[45]

Online supplementary material

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Linear regression analyses with the SI as dependent variable and dietary, body composition and inflammatory variables as independent factors.

Table S2. Subgroup analysis for the association between exposures (physical activity, android fat, and inflammatory indicators) and SI by sex and diabetes considering only variables that showed statistically significant interaction.

Figure S1. Directed acyclic graphs (DAGs). The effect of dietary (A), physical activity (B), body composition (C), and inflammatory variables on Sarcopenic Index (SI), drawn and analysed using DAGitty (www.dagitty.net). Assuming total effect, plausible confounders used in the regression analysis were as follows: physical activity (in models with dietary, body composition and inflammatory variables as independent variables), Mediterranean dietary score (in models with physical activity, body composition and inflammatory variables as independent variables), and waist circumference (in models with inflammatory variables as independent variables). Assuming direct effect (sensitivity analysis), models were additionally adjusted by plausible mediators: diabetes, NLR (in models with dietary, physical activity and body composition variables as independent variables), and waist circumference (in models with dietary and physical activity variables as independent variables).

Figure S2. Forest plot representing subgroup analysis for the association between our exposures (A. 30-second chair test, B. android fat, C. leucocyte count, D. Neutrophil-to-lymphocyte ratio) and SI by center, considering only variables that showed statistically significant interaction.

Conflict of interest

None declared.

Funding

This work was supported by the official Spanish institutions for funding scientific biomedical research, CIBER Fisiopatología de la Obesidad y Nutrición (CIBERobn) and Instituto de Salud Carlos III (ISCIII), through the Fondo de Investigación para la Salud (FIS), co-funded by the European Regional Development Fund (ERDF) (four coordinated FIS projects leaded by J.S.-S. and J.V., including the following projects: PI13/00462, PI13/01090, PI13/02184, PI14/00853, PI14/00728, PI14/01919, PI16/00501, PI16/00381, PI16/01522, PI17/00525, PI17/00532, PI17/00215, and PI17/00926), and through specific grants: the Astra Zeneca Young Investigators Award in Category of Obesity and Diabetes 2017 (D.R.); Juan de la Cierva-formación research grant (FJCI-2015-24058) of the Spanish Ministry of Economy, Industry and Competitiveness and European Social Funds (J.K.), the

'FOLIUM' programme within the FUTURMed project; and talent for the medicine within the future from the Fundación Instituto de Investigación Sanitaria Illes Balears (financed by 2017 annual plan of the sustainable tourism tax and at 50% with charge to the ESF Operational Program 2014–2020 of the Balearic Islands) (J.K.). None of the funding sources took part in the design, collection, analysis, or interpretation of the data or in the decision to submit the manuscript for publication. This work is supported by the European Research Council (Advanced Research Grant 2014–2019; agreement #340918 granted to MAM-G).

[Correction added on 21 June 2019 after first online publication: The funding information has been updated in this current version.]

References

1. Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyère O, Cederholm T, et al. Writing Group for the European Working Group on Sarcopenia in Older People 2 (EWGSOP2), and the Extended Group for EWGSOP2. Sarcopenia: revised European consensus on definition and diagnosis. *Age Ageing* 2019;**48**:16–31.
2. Arvandi M, Strasser B, Meisinger C, Volaklis K, Gothe RM, Siebert U, et al. Gender differences in the association between grip strength and mortality in older adults: results from the KORA-age study. *BMC Geriatr* 2016;**16**:201.
3. Malafarina V, Uriz-Otano F, Iniesta R, Gil-Guerrero L. Sarcopenia in the elderly: diagnosis, physiopathology and treatment. *Maturitas* 2012;**71**:109–114.
4. Roubenoff R. Sarcopenic obesity: the confluence of two epidemics. *Obes Res* 2004;**12**:887–888.
5. Wannamethee SG, Atkins JL. Muscle loss and obesity: the health implications of sarcopenia and sarcopenic obesity. *Proc Nutr Soc* 2015;**74**:405–412.
6. Kang SY, Lim GE, Kim YK, Kim HW, Lee K, Park TJ, et al. Association between sarcopenic obesity and metabolic syndrome in postmenopausal women: a cross-sectional study based on the Korean National Health and Nutritional Examination Surveys from 2008 to 2011. *J Bone Metab* 2017;**24**:9–14.
7. Lu CW, Yang KC, Chang HH, Lee LT, Chen CY, Huang KC. Sarcopenic obesity is closely associated with metabolic syndrome. *Obes Res Clin Pract* 2013;**7**:e301–e307.
8. Batsis JA, Mackenzie TA, Jones JD, Lopez-Jimenez F, Bartels SJ. Sarcopenia, sarcopenic obesity and inflammation: results from the 1999–2004 National Health and Nutrition Examination Survey. *Clin Nutr* 2016;**35**:1472–1483.
9. Hamer M, O'Donovan G. Sarcopenic obesity, weight loss, and mortality: the English Longitudinal Study of Ageing. *Am J Clin Nutr* 2017;**106**:125–129.
10. Batsis JA, Mackenzie TA, Emeny RT, Lopez-Jimenez F, Bartels SJ. Low lean mass with and without obesity, and mortality: results from the 1999–2004 National Health and Nutrition Examination Survey. *J Gerontol A Biol Sci Med Sci* 2017;**72**:1445–1451.
11. Batsis JA, Mackenzie TA, Barre LK, Lopez-Jimenez F, Bartels SJ. Sarcopenia, sarcopenic obesity and mortality in older adults: results from the National Health and Nutrition Examination Survey III. *Eur J Clin Nutr* 2014;**68**:1001–1007.
12. Ji LL. Redox signaling in skeletal muscle: role of aging and exercise. *Adv Physiol Educ* 2015;**39**:352–359.
13. Guescini M, Tiano L, Genova ML, Polidori E, Silvestri S, Orlando P, et al. The combination of physical exercise with muscle-directed antioxidants to counteract sarcopenia: a biomedical rationale for pleiotropic treatment with creatine and coenzyme Q10. *Oxid Med Cell Longev* 2017;**2017**:1–19.
14. Franceschi C, Garagnani P, Parini P, Giuliani C, Santoro A. Inflammaging: a new immune-metabolic viewpoint for age-related diseases. *Nat Rev Endocrinol* 2018;**14**:576–590.
15. Franceschi C, Garagnani P, Vitale G, Capri M, Salvioli S. Inflammaging and 'garb-aging'. *Trends Endocrinol Metab* 2017;**28**:199–212.
16. Cantero I, Abete I, Babio N, Arós F, Corella D, Estruch R, et al. Dietary Inflammatory Index and liver status in subjects with different adiposity levels within the PREDIMED trial. *Clin Nutr* 2017;**37**:1736–1743.
17. Chan RSM, Yu BWM, Leung J, Lee JSW, Auyeung TW, Kwok T, et al. How dietary patterns are related to inflammaging and mortality in community-dwelling older Chinese adults in Hong Kong—a prospective analysis. *J Nutr Health Aging* 2019;**23**:181–194.
18. Ticinesi A, Meschi T, Lauretani F, Felis G, Franchi F, Pedrolli C, et al. Nutrition and Inflammation in older individuals: focus on vitamin D, *n*-3 polyunsaturated fatty acids and whey proteins. *Nutrients* 2016;**8**:186.
19. Mithal A, Bonjour JP, Boonen S, Burckhardt P, Degens H, El Hajj Fuleihan G, et al. Impact of nutrition on muscle mass, strength, and performance in older adults. *Osteoporos Int* 2013;**24**:1555–1566.
20. Martínez-González MA, Buil-Cosiales P, Corella D, Bulló M, Fitó M, Vioque J, et al. Cohort profile: design and methods of the PREDIMED-Plus randomized trial. *Int J Epidemiol* 2018; https://doi.org/10.1093/ije/dyy225. (in press).
21. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International. *Circulation* 2009;**120**:1640–1645.
22. Peters DM, Wacker WK, Davis CE, Shapiro MD, Ergun DL. Dual-energy X-ray absorptiometry for quantification of visceral fat. Kaul S, Rothney MP. *Obesity (Silver Spring)* 2012;**20**:1313–1318.
23. Lee WJ, Liu LK, Peng LN, Lin MH, Chen LK, ILAS Research Group. Comparisons of sarcopenia defined by IWGS and EWGSOP criteria among older people: results from the I-Lan longitudinal aging study. *J Am Med Dir Assoc* 2013;**14**:528.e1–528.e7.
24. Jang BY, Bu SY. Total energy intake according to the level of skeletal muscle mass in Korean adults aged 30 years and older: an analysis of the Korean National Health and Nutrition Examination Surveys (KNHANES) 2008–2011. *Nutr Res Pract* 2018;**12**:222–232.
25. Chung JY, Kang HT, Lee DC, Lee HR, Lee YJ. Body composition and its association with cardiometabolic risk factors in the elderly: a focus on sarcopenic obesity. *Arch Gerontol Geriatr* 2013;**56**:270–278.
26. Fernández-Ballart JD, Piñol JL, Zazpe I, Corella D, Carrasco P, Toledo E, et al. Relative validity of a semi-quantitative food-frequency questionnaire in an elderly Mediterranean population of Spain. *Br J Nutr* 2010;**103**:1808–1816.
27. Moreiras O, Carvajal A, Cabrera L, Cuadrado C. Tablas de composición de alimentos "Food Composition Tables" Pirámide. Madrid, Spain. 2005. https://scholar.google.com/scholar?cluster=11259399109894736650&hl=en&oi=scholarr#0. Accessed May 26, 2015.
28. Mataix Verdú JMAM. In de Granada U, ed. *Tabla de Composicion de Alimentos [Food composition tables]*. Granada, Spain: Universidad de Granada; 2003.
29. Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean diet and survival in a Greek population. *N Engl J Med* 2003;**348**:2599–2608.
30. Molina L, Sarmiento M, Penafiel J, Donaire D, Garcia-Aymerich J, Gomez M, et al. Validation of the Regicor short physical activity questionnaire for the adult population. *PLoS ONE* 2017;**12**:1–14.
31. Rosique-Esteban N, Díaz-López A, Martínez-Gozález M, Corella D, Goday A, Martínez JA, et al. Leisure-time physical activity, sedentary behaviors, sleep and cardiometabolic risk factors at baseline in the

PREDIMED-PLUS intervention trial: a cross-sectional analysis. *PLoS ONE* 2017;**12**:1–19.

32. Ainsworth BE, Haskell WL, Herrmann SD, Meckes N, Bassett DR Jr, Tudor-Locke C, et al. 2011 Compendium of physical activities: a second update of codes and MET values. *Med Sci Sports Exerc* 2011; **43**:1575–1581.
33. Ruiz Comellas A, Pera G, Baena Díez JM, Mundet Tudurí X, Alzamora Sas T, Elosua R, et al. Validation of a Spanish short version of the Minnesota Leisure Time Physical Activity Questionnaire (VREM). *Rev Esp Salud Publica* 2012;**86**:495–508.
34. Jones CJ, Rikli RE, Beam WC. A 30-s chair-stand test as a measure of lower body strength in community-residing older adults. *Res Q Exerc Sport* 1999;**70**:113–119.
35. Textor J, van der Zander B, Gilthorpe MS, Liśkiewicz M, Ellison GT. Robust causal inference using directed acyclic graphs: the R package "dagitty.". *Int J Epidemiol* 2016;**45**:1887–1894.
36. Schleicher RL, Carroll MD, Ford ES, Lacher DA. Serum vitamin C and the prevalence of vitamin C deficiency in the United States: 2003–2004 National Health and Nutrition Examination Survey (NHANES). *Am J Clin Nutr* 2009;**90**:1252–1263.
37. Traber MG, Buettner GR, Bruno RS. The relationship between vitamin C status, the gut–liver axis, and metabolic syndrome. *Redox Biol* 2018;**21**:101091.
38. Ralston JC, Lyons CL, Kennedy EB, Kirwan AM, Roche HM. Fatty acids and NLRP3 inflammasome-mediated inflammation in metabolic tissues. *Annu Rev Nutr* 2017; **37**:77–102.
39. Tyrovolas S, Koyanagi A, Olaya B, Ayuso-Mateos JL, Miret M, Chatterji S, et al. Factors associated with skeletal muscle mass, sarcopenia, and sarcopenic obesity in older adults: a multi-continent study. *J Cachexia Sarcopenia Muscle* 2016;**7**:312–321.
40. Kalinkovich A, Livshits G. Sarcopenic obesity or obese sarcopenia: a cross talk between age-associated adipose tissue and skeletal muscle inflammation as a main mechanism of the pathogenesis. *Ageing Res Rev* 2017;**35**:200–221.
41. Lim HS, Park YH, Suh K, Yoo MH, Park HK, Kim HJ, et al. Association between sarcopenia, sarcopenic obesity, and chronic disease in Korean elderly. *J Bone Metab* 2018;**25**:187–193.
42. Levine ME, Crimmins EM. The impact of insulin resistance and inflammation on the association between sarcopenic obesity and physical functioning. *Obesity (Silver Spring)* 2012;**20**:2101–2106.
43. Rubio-Ruiz ME, Guarner-Lans V, Pérez-Torres I, Soto ME. Mechanisms underlying metabolic syndrome-related sarcopenia and possible therapeutic measures. *Int J Mol Sci* 2019;**20**: pii: E647.
44. Calvani R, Marini F, Cesari M, Buford TW, Manini TM, Pahor M, et al. Systemic inflammation, body composition, and physical performance in old community-dwellers. *J Cachexia Sarcopenia Muscle* 2017;**8**:69–77.
45. von Haehling S, Morley JE, Coats AJS, Anker SD. Ethical guidelines for publishing in the Journal of Cachexia, Sarcopenia and Muscle: update 2017. *J Cachexia Sarcopenia Muscle* 2017;**8**:1081–1083.